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Original Research

Antimicrobial Activity of *Lantana camara* Essential Oil

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Lantana camara leaves were harvested and the essential oils of the leaves were extracted through steam distillation. Some physico-chemical properties of the oil like density, solidifying point, pH value and uv-absorption spectrum in ethanol were determined. Antimicrobial activity of the oil was assayed using organisms like *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger.* The oil was incorporated into petrolatum to form ointment. Effectiveness of the ointment on the susceptible organisms was checked. The ointment was given to human volunteers. The yield was 0.96% (W/W). The density, boiling point and solidifying point of the oil were 0.8685g/l, 118 °C and – 0.5 °C respectively while the viscosity is 141 centipoise. The pH value and acid value were 2.4 and 8.976, respectively while the λ_{max} was at 380 nm. The oil had antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* among the tested organism but showed no anti-fungal property. The ointment had antibacterial activity and no skin reaction within the experimental condition and the scent of the oil was strong even after 24 hrs from the time of the application. The oil can be incorporated into creams and other topical formulations.

INTRODUCTION

There is increase in demand for essential oils for pharmaceutical and allied uses. This has led to rewarding studies and researches on many plants, which yield essential oils that have pharmaceutical uses and other applications[1].

Lantana camara is an annual plant of the genus Lantana, which belongs to the verbenaceae family. It is 2-5 m tall and is popularly known as yellow sage. The flowers are yellow, later turning orange then red and remain on the auxiliary inflorescence for three days. The flowers produce nectar. The fleshy drupe is 3-6 mm in diameter containing 1-2 seeds. Fruits mature rapidly and change colour from dark green to black. The leaves are evergreen. *Lantana camara* is a poisonous plant[2,3].

From ethyl acetate extract, campestral, stimasterol, β-sitosterol, β-sitosterol-3-0-β-D-glycoside were isolated[4-7]. Oleanolic acid, Lantadene β, β-angeloyl-oxylantanolic acid, betulinic acid, Pomolic acid, Lantadene A and triterpenoids have also been isolated from the stems of the yellow flowering taxa of *Lantana camara* [8-11].

The plant has anti-catarrah, antiviral, anti-tumor, emmenagogue and mycolytic activities. It may help with bronchitis and asthma[10]. It is employed in traditional medicine in China and some West African countries as antimicrobial and anti-fungal agents[6,12].

The aim of this work was to isolated the essential oil of *Lantana camara* and determine its physicochemical properties as well as its anti-microbial activity alone and in ointment formulation.

Materials and Methods

Plant and reagents

Fresh leaves of *Lantana camara L (Verbenaceae)* were harvested from Nsukka, Enugu state in June 2004 and authenticated by Mr. J. M. C. Ekekwe of the Department of Botany, University of Nigeria, Nsukka. Dimethylsuphoxide (DMSO) and ethanol were sourced commercially and were products of BDH England. Sabouraud's broth, nutrient agar and peptone water were products of Merck. Other reagents used were standard laboratory reagents.

Microorganisms

The microorganisms used were obtained from the Department of Pharmaceutics, University of Nigeria, Nsukka, where they are maintained as standard cultures. The organisms used were *Pseudomonas aeruginosa* (NCTC 6750), *Salmonella typhi* (Laboratory strain), *Staphylococcus aureus* (NCTC 3761), *Bacillus subtilis*, (NCTC 6750), *Escherichia coli, (NCTC 9001), Aspergillus niger* (laboratory strain) and *Candida albicans* (laboratory strain).

Extraction and characterization of the oil

The plant was harvested early in the morning and was used immediately. The oil was extracted through steam distillation. The colour and scent of the oil were noted. The density, viscosity, boiling point, solidifying point, pH value of the oil was determined[12]. An ethanolic solution of the oil was scanned in SP8-100 UV- spectrophotometer a product of Pye Unicam, England and the spectrum was recorded.

Screening for Anti-microbial Activity

The oil was solubilised with DMSO and diluted with water. The concentrations of the solutions were noted. Agar diffusion method was used to determine the sensitivity of the organisms to the oil[14]. Bacterial and fungal cultures were prepared in nutrient agar and Sabouraud's broth media in plate form respectively. A cork borer (12 mm) was used to bore wells into the plates. One milliliter of 5 mg/ml of the solution was introduced into the hole bored into the plate containing each organism. The media were incubated for 24 hr at 37°C and for 48 hr at 27° C for bacteria and fungi, respectively, after which the zones of inhibitions were measured.

The minimum inhibitory concentration (MIC) was determined through agar serial dilution method[15]. Serial concentrations 7.5, 5.0, 2.5, 1.25, 0.75 mg/ml of essential solution were prepared and 1.0 ml of each was introduced into the hole in the prepared agar plate. The plates were incubated as stated above and inhibition zone diameters (IZD) were measured and the squares of IZD $(IZD)^2$ were calculated . The MIC was obtained in each case from the intercept of the logarithmic concentration (log conc.) axis of graph of $(IZD)^2$ against log conc. Nystatin® and chloromycetin® were used as standards.

Preparation and evaluation of ointment with the oil:

An ointment containing 5% (w/w) of the oil in petrolatum was prepared. A dispensing balance was zeroed with a sterile 50 ml beaker on the weighing pan. Known quantities of the ointment were introduced into the holes made on the agar culture media. The inoculated plates were incubated at 37°C for 24 hr and the zones of inhibition were measured. Chloramphenicol ointment was used as a control.

The ointments were given to 15 human volunteers. Five applied it once a day; another five applied it twice a day; and the remaining five applied it three times a day. The time duration of the scent was noted and reactions on the skin were equally noted.

RESULTS AND DISCUSSION

The steam distillation gave light pale yellow liquid, which was 0.96% (W/W) of the fresh leaves. The yield was very poor; probably because the leaves were not harvested at flowering stage. For most plants, the concentration of the essential oil is higher at the flowering stage. The oil had a sweet smell, with density, boiling point and solidifying point values 0.8685g/l, 118°C and –0.5°C, respectively. Its viscosity was 141 centipoise. The low solidifying point indicates that the oil will remain a liquid at room temperature and the high boiling point means that the sweet smell will last when sprayed. The pH value of 2.4 of the oil indicates the presence of acidic compounds which was supported by the acid value of 8.976. This means that the oil contains volatile fatty acids. The high viscosity of the oil indicates the presence of conjugated molecules a fact supported by its λ-max value of 380 nm. The occurrence of peaks at 200 nm and 350 nm however show that the oil contains both saturated and unsaturated compounds. The spectrum is obtained in ethanol and is as shown as Fig 1.

Essential oils of *Lantana camara* had an antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* among the tested organisms. It had no anti-fungal action. The detail of the result is shown as tables 1 and 2. The minimum inhibitory concentrations were obtained as the antilog of the intercepts of the plots of log. concentration against square of inhibition zone diameter. When compared with chloromycetin, the oil is 143 time less potent on *Bacillus subtilis* and 620 times less potent on *Staphylococcus aureus*. The activity of the oil on *Bacillus subtilis* is more than that on *Staphylococcus aureus*.

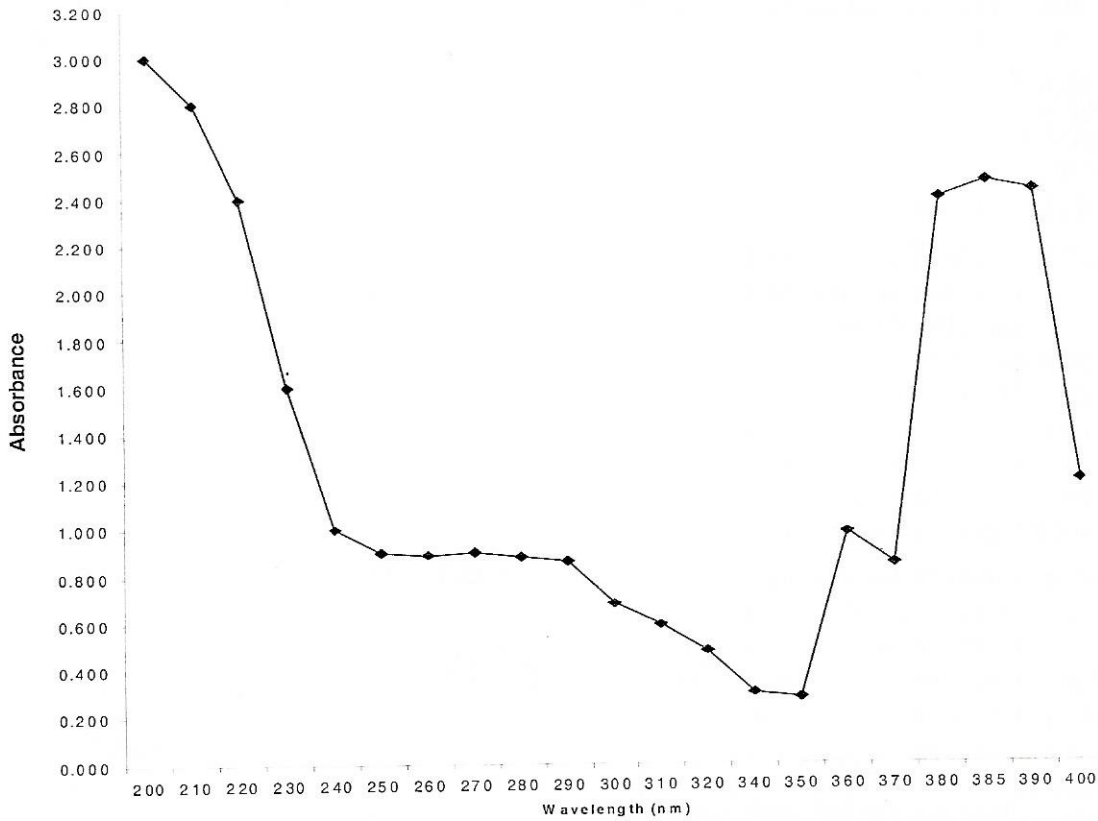

Fig. 1: Absorption Spectrum of *Lantana camara* oil in ethanol

Table 1. Anti-microbial activity of the oil

Organism	Activity of the Oil	Inhibition zone diameter (IZD) cm
Staphylococcus aureus	Active	2.0
Bacillus subtilis	Active	4.4
Escherichia coli	Not active	--
Salmonella typhi	Not active	--
Pseudomonas aeruginosa	Not active	--
Candida albicans	Not active	--
Aspergillus niger	Not active	--

Table 2. The minimum inhibitory concentration (MIC) of the oil on the sensitive organism.

Organism	Oil (mg/ml)	Gentamycin® (mg/ml)
S. aureus	108.56	0.1750
Bacillus subtilis	6.23	0.0435

The ointment blended well with petrolatum and had a comparable activity with chloramphenicol ointment when tested in-vitro. At the tested concentration of the oil in the ointment, the scent lasted for more than a day and showed no skin reaction or irritation when applied to the body as confirmed from volunteers who applied the oil once a day. Volunteers who

applied the ointment twice or thrice a day had no skin reaction within the experimental conditions.

From the findings, the oil can be incorporated in soaps, creams and other topical formulations as a scent or as an antibacterial agent. A little quantity of the oil can achieve the expected effects.

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